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Gut Reaction to Wnt Signaling in Worms

Minireview

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The demonstrations in two papers in this issue of *Cell* (Rocheleau et al., 1997; Thorpe et al., 1997) of the involvement of a Wnt pathway in very early embryogenesis in Caenorhabditis elegans provides another significant step toward the ambitious but realistic goal of understanding all of the basic strategies used to control embryogenesis in this model organism. At the same time, they challenge some of the prevailing models of Wnt signaling, suggesting that interactions among Wnt pathway components may vary in different developmental processes. With these papers, as well as the earlier reports on Wnt pathway genes *lin-44*, *lin-17*, and *pop-1* (Herman et al., 1995; Lin et al., 1995; Harris et al., 1996; Sawa et al., 1996) and new studies on Wnt pathway genes reported in recent meetings, worm breeders have become a significant force in the army of Wnt researchers. They have also illustrated how different systems can provide important new complementary insights.

Signaling at the Four–Cell Stage Polarizes a Blastomere

The establishment of tissue specificity in the early C. elegans embryo is directed by the combination of asymmetric cell divisions guided by intrinsic mechanisms and cell–cell interactions (reviewed by Schnabel and Priess, 1997). The fertilized egg, polarized by the entrance of the sperm, divides unevenly and generates an unpolarized cell AB and a polarized cell P_1. Some evidence suggests that a signal from P_1 to AB specifies the anterior–posterior differences of AB descendants. During the second round of divisions, the P_1 cell again generates an unpolarized cell EMS and a polarized daughter cell P_2, whereas AB generates two cells (ABp and ABa) with equal developmental potential. While the cell fate of P_2 continues to be dictated, at least in part, by intrinsic factors, the cell division patterns and differential fates of ABp and EMS are instructed by P_2-originated cell signaling at the four-cell stage (Figure 1). A signal from P_2 to ABp breaks the equivalence between ABp and Aba, establishing the dorsal/ventral, differential fates of their descendants. This signaling event is mediated by the APX-1 (Delta-like) and the GLP-1 (Notch-like) proteins. A second signal from P_2 polarizes its sister cell, EMS, which results in the two daughters of EMS, E and MS, having strikingly different cell fates. This P_2-EMS signaling event has now been shown to involve a Wnt pathway.

During normal embryogenesis, the descendants of MS primarily generate mesodermal tissues, such as pharyngeal tissue and body wall muscle, while the descendants of E are endodermal cells that make the intestine (gut). E cell fate depends upon an inductive signal originating outside of EMS, since when an EMS blastomere is isolated and allowed to develop alone, both of its daughter cells differentiate like MS and no longer produce gut (Goldstein 1993). Further nongenetic manipulations of cultured embryonic cells have demonstrated that P_2 is the source of this signal and that the position of P_2 relative to EMS determines which daughter of EMS produces gut.

mom *Genes Define a Wnt Pathway That Induces Cell Polarity and Gut Differentiation*

The pioneering studies using cultured blastomeres were followed by classical genetic screens for mutant embryos exhibiting the gutless phenotype expected from disrupting P_2-EMS signaling (E transformed to MS) (Rocheleau et al., 1997; and Thorpe et al., 1997). The two research groups isolated 29 such mutations that define five *mom* (*mo*re *m*esoderm) genes. Three of the genes have been cloned and found to encode components in the Wnt signal transduction pathway. *mom-2* and *mom-5* encode a Wnt-like molecule and a Frizzled (Fz)-like receptor, respectively. *mom-1* encodes a protein similar to Drosophila Porcupine (Porc), a protein required for Wnt protein processing and secretion (Rocheleau et al., 1997). Using cultured chimeric, partial embryos, Thorpe et al. (1997) determined that *mom-1*, *mom-2*, and *mom-3* (uncloned) act in the signaling P_2 cell, and *mom-4* (uncloned) acts in the responding EMS cell. A function for *mom-5* in EMS is assumed based on its Fz-like structure.

Rocheleau et al. (1997) have used RNA-mediated interference (RNAi) to disrupt the expression of a C. elegans *armadillo* homolog (*wrm-1*) and an APC-related gene (*apr-1*). The RNAi method, which involves injecting either sense or antisense RNA of a specific gene into

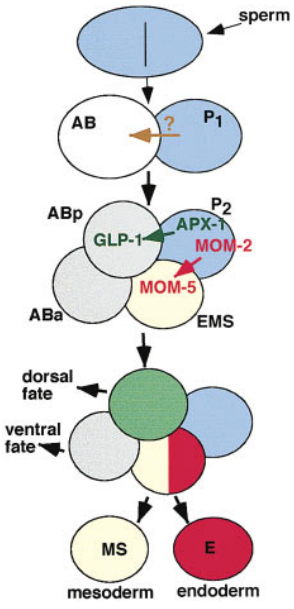

Figure 1. Cell–Cell Signaling in Four–Cell Embryo of C. elegans

See the text for explanation and Schnabel and Priess (1997) for a detailed review.

the gonad of C. elegans hermaphrodites, has recently been shown in many laboratories to generate loss-of-function mutant phenotypes (refs. in Rocheleau et al., 1997). For example, injections of RNA of other Wnt pathway genes like *mom-2*, *mom-5*, and *pop-1* result in similar phenotypes as those of mutations in these genes. RNAi is now commonly used to probe functions of cloned genes in early developmental events. How RNAi disrupts gene activity is currently not understood, although it has been observed in several cases that the protein is not expressed in RNAi-treated embryos. Injections of *wrm-1* or *apr-1* RNA, which presumably suppress the endogenous genes' activities, cause the same gutless phenotype as that of *mom* mutants, suggesting both genes act positively in this *wnt*-mediated signaling pathway. In order to confirm these RNAi observations, and to study further the functions of *wrm-1* and *apr-1*, it will be still desirable to have true genetic mutations in these genes.

One other gene also involved in specifying cell fates of E and MS is *pop-1* (Lin et al., 1995). *pop-1* mutants have a phenotype opposite to that of the *mom* mutants in gut induction, as both E and MS cells adopt an E-like fate and produce gut tissue. *pop-1(+)* activity thus normally suppresses the E cell fate. *pop-1* encodes an HMG-domain protein similar to the TCF and LEF-1 proteins discovered in vertebrates and more recently in Drosophila as downstream factors in the Wnt pathway (Figure 2). In double mutants, the *pop-1* mutant phenotype (extra gut) is completely epistatic to the gutless phenotype of *mom*, *wrm-1*, and *apr-1* mutants. In wild-type worms, POP-1 protein is localized with higher intensity in MS nuclei than in E nuclei, and this differential staining is eliminated in *mom-2* (Wnt) or *wrm-1* (Arm) mutants. These results suggest that the Wnt signaling pathway functions upstream of POP-1 to downregulate its level or activity in the E cell nucleus (Rocheleau et al., 1997; Thorpe et al., 1997). Since the mother cell EMS is already polarized for gut potential prior to its division, POP-1 distribution is likely already polarized in EMS, and the difference is then carried to the daughters.

Different Models for Functions of β-catenin, TCF, and APC Gene Families

In recent years, major advances have been made in understanding Wnt signaling pathways in Drosophila and in vertebrates that control a variety of cell differentiation and pattern formation decisions (e.g., reviewed by Peifer, 1996; Moon et al., 1997; Nusse 1997). A prevailing model of the actions of downstream Wnt-signaling components is depicted in Figure 2A and 2C. In the absence of a Wnt signal, β-catenin/Arm level is low due to degradation promoted by GSK-3 kinase and APC. In the presence of a Wnt signal, an Fz-like receptor is activated and it, in turn, activates Dishevelled (Dsh). Dsh then inactivates the GSK-3 kinase, resulting in a high level of β-catenin. β-catenin then interacts with TCF/LEF-1. The complex translocates to the nucleus to activate transcription of Wnt-responsive genes. The evidence for the positive role of TCF/LEF-1 in Wnt signaling is summarized below (see Nusse, 1997, for references). TCF and LEF-1 contain an HMG-box that binds to DNA and causes bending of the helix. Binding between TCF and β-catenin appears to promote β-catenin translocation to the nucleus, and β-catenin appears to be a coactivator when forming the complex with TCF. In functional tests using Xenopus embryos, injection of *LEF-1* RNA into ventral blastomeres induces the formation of a second body axis as does Wnt. More convincing data supporting a positive role of TCF in Wnt signaling comes from the more recent genetic work on the Drosophila gene named *pangolin* (*pan*) or *dTCF* (Brunner et al.,

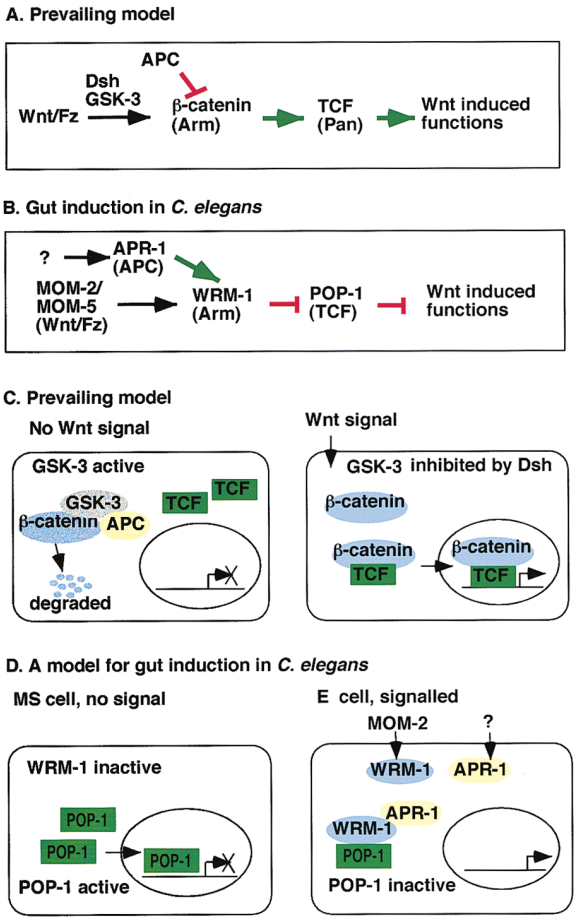

Figure 2. Comparison of Two Models for the Actions of Wnt Signaling Pathway Components

(A and C) A currently prevailing model for genetic (A) and molecular (B) functions of downstream Wnt pathway components in Drosophila and vertebrates (Peifer, 1996; Moon et al., 1997; Nusse, 1997). See text for discussion. The role of APC-like protein in Drosophila *wingless* pathway is currently not clear.

(B and D) A Model for the genetic (B) and molecular (D) functions of downstream Wnt pathway components during gut induction in C. elegans (based on Rocheleau et al., 1997, and Thorpe et al., 1997). APR-1, an APC homolog, is proposed to act positively and in parallel to MOM-2 (Wnt) and MOM-5 (Fz). WRM-1, an Arm/β-catenin homolog, is proposed to down-regulate POP-1, a TCF homolog. POP-1 suppresses E-specific functions in the nucleus in the absence of Wnt signaling. Since induction of gut differentiation (E cell fate) occurs in the mother cell prior to the cell division, the cytoplasmic actions of this pathway, including activation of WRM-1 and even distribution of POP-1 protein in the cytoplasm, are likely to occur in the mother cell to polarize it. The nuclear function of POP-1 is likely to occur after the cell division. For convenience, Figure 2D depicts this whole process in two separated cells. Much about the molecular actions of these C. elegans proteins is speculated based on C. elegans genetics and known biochemical functions of vertebrate proteins.

1997; van de Wetering et al., 1997). Loss-of-function mutations in *pan* result in phenotypes similar to that of loss of *wingless* (*wg*) functions. Since *pan* mutations suppress the phenotype of constitutively activated *arm*, Pan (TCF) is thus required for Arm (β-catenin) to transduce the Wg signal.

The genetic properties of *pop-1* (TCF) clearly do not fit the above model (Figure 2). In C. elegans, *wrm-1* (Arm) inactivates *pop-1* (TCF), whereas in Drosophila, *arm* activates *pan* (TCF). Such a contradiction suggests that the same proteins may execute different roles under different developmental circumstances or different subtypes of the protein families may function differently. It could be speculated that in the case of C. elegans gut induction, POP-1 protein, either by itself or by forming a complex with another unknown protein, functions to repress transcription of genes specific for the gut fate. The Wnt signal from the P_2 cell may activate WRM-1 in the posterior half of EMS, and binding of WRM-1 to POP-1 would then form a nonfunctional, perhaps cytoplasmic, complex. The E cell would then differentiate into gut by a default mechanism (Figure 2D). Such a scheme may be consistent with some work done in other systems. In Xenopus, a mutant XTCF3 with its β-catenin binding domain deleted was shown to suppress normal axis formation (Molenaar et al., 1996). Merriam et al. (1997) also showed that cytoplasmically anchored plakoglobin (another vertebrate homolog of Arm) can nevertheless induce a phenotype similar to that induced by Wnt.

In the vertebrate model, APC plays a negative role in Wnt signaling as it functions to promote β-catenin degradation by forming a protein complex with β-catenin and GSK-3. Mutations in APC block such down-regulating processes in mammalian cells, resulting in a high level of cytoplasmic β-catenin activity (Peifer 1996). In contrast, a positive role has been proposed for C. elegans *apr-1* (APC) in gut induction (see above, Figure 2B and 2D). A positive role of APC may also be suggested by the unexpected result in Xenopus (Vleminckx et al., 1997) that APC induces a duplication of the body axis similar to that induced by Wnt. Given that this effect appears to require cytosolic β-catenin, APC may act together with β-catenin to promote signaling (see Peifer, 1996). APC thus may also exert different functions under different circumstances. In C. elegans, APR-1 (APC) may act with WRM-1 (Arm) to down-regulate POP-1 (TCF) (Figure 2D).

Multiple Signaling Input in Gut Induction

One striking feature of the genes involved in worm gut induction is the difference in the penetrance of their mutant phenotypes (Rocheleau et al., 1997; Thorpe et al., 1997). Although lesions in many *mom* mutants suggest they are null or severe loss-of-function mutations, the percentage of the mutant embryos with the gutless phenotype is low for some genes. For example, less than 10% of the *mom-5* (Fz) mutant embryos and only 26% of *apr-1(RNAi)* embryos lack endoderm, suggesting that there are other signaling molecules that can mediate P_2-EMS induction. Since disrupting both the *apr-1* (APC) and *mom-5* (Fz) genes causes a highly penetrant gutless phenotype, *apr-1* may act in parallel to *mom-5* to induce the E cell fate. These two signaling branches may converge on the *wrm-1* (Arm) gene since disrupting *wrm-1* gene activity results in a completely penetrant gutless phenotype (Rocheleau et al., 1997; Figure 2B and 2D).

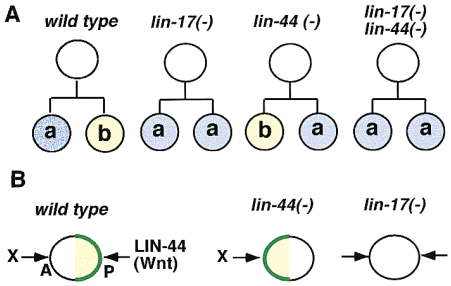

Figure 3. Role of *lin-44* (Wnt) and *lin-17* (Fz) in Determining Cell Polarity and Polarity Orientation

(A) Summary of *lin-17* and *lin-44* mutant phenotypes. (a) and (b) represent two cells with distinct lineages and differential fates.

(B) One possible model for roles of *lin-44* (Wnt) and *lin-17* (Fz) in determining the asymmetry of certain cells' (B and T cells) divisions in C. elegans (proposed by Sawa et al., 1996). Green half-circles indicate activated LIN-17 receptors. X, denotes an unknown Wnt-like signal. See the text for explanation.

Another puzzling phenomenon is that *mom-5* (Fz) mutants have a much lower penetrance of the phenotype (5% for allele *zu193*) than do *mom-2* (Wnt) mutants (39% for allele *ne141*). The double mutant with both *zu193* and *ne141* is surprisingly similar to the weaker *mom-5* single mutant. This result suggests that the MOM-5 (Fz) receptor has a negative role in the absence of the MOM-2 (Wnt) signal (e.g., by responding to a different signal that antagonizes WRM-1 activity). Wnt signaling processes, like many other signaling pathways, are likely to be networks that involve cross-talk and feedback loops, rather than simple linear cascades.

Multiple Decisions Controlled by Multiple Wnt Signaling Pathways

wnt genes and several components in the *wnt* pathway exist as multigene families in vertebrates, Drosophila and C. elegans. For example, there are at least 16 *wnt*-like and eight *fz*-like genes in the mouse (Moon et al., 1997) and at least five *wnt*-like and four *fz*-like genes in C. elegans. Multiple genes also appear to encode Dsh and Arm homologs. Although some family members might have redundant roles, in many cases, functional studies have indicated that different members function in distinct developmental decisions. Current work on *mom-2* (Wnt) and *mom-5* (Fz) in C. elegans has defined developmental functions that are distinct from those of the previously described *lin-44* (Wnt) and *lin-17* (Fz) genes (Herman et al., 1995; Harris et al., 1996; Sawa et al., 1996).

lin-17(Fz) mutations disrupt the asymmetry of cell division in unrelated cells in many different tissues (Figure 3A). *lin-17* (Fz) appears to function in the mother cell to polarize the cell and thus plays a role similar to that of *mom-5* (Fz) in the induction of EMS polarity. However, *lin-44* (Wnt) mutations cause reversals, but not elimination, of the polarity of division of a subset of those cells affected by *lin-17* (Fz) mutations (Figure 3A). To explain the difference in the phenotype by these two genes, Sawa et al. (1996) proposed the existence of additional signal(s) for the *lin-17* receptor (Figure 3B). In their

model, LIN-17 would be activated only in the posterior half of the cells, presumably by the directional signal of LIN-44 (Wnt). However, LIN-17 receptor would also be activated in the anterior half of the cells by another Wnt-like signal (X). The effect of this unidentified anterior signal is proposed to be overridden by the LIN-44 (Wnt) signal. When the *lin-17* (Fz) gene is mutated, the asymmetry of division and the major difference between the two daughter cells are abolished. The role of *lin-44* in this model is consistent with its cell-nonautonomous function and its expression in cells that are located posterior to the cells receiving the signal (Herman et al., 1995).

Many *wnt* genes (e.g., the *wingless* gene in the fly) function in multiple developmental events, and this may also be the case for the *mom* genes. Some *mom* genes appear to function to orient mitotic spindles in many blastomeres during early development. For example, *mom-1* (Porc), *mom-2* (Wnt), *mom-5* (Fz) and *mom-3* (uncloned) mutants all have fully penetrant defects in mitotic spindle orientation of the ABar cell, a granddaughter of AB (Rocheleau et al., 1997; Thorpe et al., 1997), a phenotype that suggests a role for the Wnt signaling in the regulation of cytoskeletal organization during mitosis. Since disruption of *wrm-1* (Arm), *apr-1* (APC), *mom-4* (uncloned), and *pop-1* (TCF) does not result in a mitotic spindle phenotype in ABar, this suggests that the *wnt* pathway takes a different turn at a point somewhere between the receptor (MOM-5) and WRM-1. Since normal spindle orientation in early blastomeres does not depend upon embryonic transcription, it is likely that the Wnt signal does not involve a DNA target (Rocheleau et al., 1997). This process could have some similarity to tissue polarity determination in Drosophila where *fz*, *dsh*, and *rhoA*, but not other downstream factors of the *wnt* pathway, were found to play roles (Drasnow et al., 1995; Strutt et al., 1997).

Conclusions

Current studies using C. elegans genetics are expected to generate more exciting results about the functions of Wnt signaling pathways in various developmental events. While the research projects will continue to aim at understanding the cellular and molecular mechanisms of cell signaling events in directing development, some cell signaling events such as the P_2-to-EMS signaling will also be excellent assay systems to dissect the Wnt signal transduction pathways. Not only do these genetic and molecular analyses increase our knowledge of the functions of the known components of the Wnt pathways, but they might also reveal new components of the pathways, as the cloning of *mom-3* and *mom-4* is likely to prove.

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